Part III: Thesis Description Sheet

A. Title

STUDY OF POST ANTHESIS LEAF HEALTH AS POTENTIAL YIELD DETERMINANT IN EARLY GENERATION TRIAL OF WHEAT IN RAMPUR, CHITWAN

B. Objectives

General

- To identify genotypic and environmental factors associated with yield formation during post-anthesis stage in relation to leaf health indicators.
- To describe variation associated with enhanced leaf health for complementing current state of knowledge to better prepare plan wheat breeding programs.

Specific

- To isolate spatial features that predispose leaf to various health states during post-anthesis.
- To identify plant traits enhancing post-antheis leaf longevity.
- To identify potential genotypes with favorable variation for enhanced post-antheis leaf health.
- To study reliability of yield prediction using post-antheis terminal leaves associated traits.

C. Summary (Not more than 200 words)

Wheat is often times credited for having had a central role to the beginning of agriculture (Harlan, 1981). With the necessity to feed burgeoning population, current wheat breeding programs frequently target yield component traits rather than the yield per se, with a hope to have positive impact on yield. In the search for candidate traits leading to improved photosynthetic potency, effects of leaf health in yield and component traits, will be studied. It is established that correctly defining fields' spatial pattern and accounting for those will lead to improved accuracy in estimation of yield and component traits. Current study seeks to employ grid design in order to recover inter-block information from the trial where a large number of unreplicated treatment entries will be augmented in replicated blocks of check varieties. Observations will be made of traits that characterize leaf health status during post-antheis period of wheat growth. Since a major fraction of final grain weight is a function of genotypic and environmental factors during crop's grain filling period, these factors will be explored through potential yield determining traits, expressed in diverse germplasm of spring grown wheat genotypes (108 genotypes in total). Mainly, the stay green trait (expressed as time dependent decline of leaf chlorophyll; measured as SPAD decline), a visual assay of traits conferring potential disease resistance (expressed in level of necrosis and chlorosis of the leaf), the leaf area index (LAI), and other traits associated with the canopy structure that potentially predispose leaf photosynthetic ability to environmental stressing factors, all of which implied in yield determination will be observed. Linear mixed effects models will be fitted by restricted maximum likelihood estimation method to the responses, and the yield prediction will be accompanied by growth stage based interpretation. Finally comparison across the distinct classes of adapted and early generation genotypes will be made in an attempt to suggest for varietal improvement plans.

D. Introduction

Agricultural statistics published by the Ministry of Agriculture Development states that the country imported food items worth around 10 billion NPR in the year 2015/016 (Nepal, 2014). Despite an appreciable government's commitment to agriculture, as evident from the recent trends in fiscal year allocation of budget for agriculture sector, a forerunner indicative of ADSs' vision to achieve food self-sufficiency status is yet to be realized (Nepal, 2017).

The bread Wheat(Triticum aestivum L.), a crop that ranks first globally in cereal protein value, and that provides for majority of staple carbohydrates in the developed world, is one among a few major food crops grown worldwide. It has one of the largest known genome size among cultivated species. It is a hexaploid with a genome thought to be composed of two or more distinct species' genomes following a series of allopolyploidization events in the past. Bread wheat has been traced to be composed of T. urartu(A), the Sitopsis section of Aegilops(B), and Aegilops tauschii(D) genomes (Dvorak et al. , 1998).

Early testing is a problem of how to optimize the use of resources. For a plant breeding program, having to reliably discriminate between large numbers of genetic resources, those generated from crosses or those collected from diverse domain, is what generally entails early testing. The outcome of any planned crossing programs and it's advanced generations–the seed propagule, has to qualify a test or more to exhibit it’s valuable, and to deliberately proceed to further generations, eventually in order to make its way of being released as a cultivar.

Crop breeding programs frequently target yield component traits rather than the yield per se, with the hope to have positive effect on yield (Slafer, 2003). Among a large inventory of candidate traits, only a few can actually be reliably used in planned improvement programs. The problem with candidate traits usually is that underlying mode of trait influencing the yield is not well established, and even if the biological pathway is well established, problem of unstably inherited underlying variation remains (Saadalla, 1994); even acclaimed yield component traits such as number of spikes per plant have been asserted as not being an effective selection criterion (McNeal et al., 1978). Thus, the experiments fail to produce expected results under differing field conditions (Okuyama, Federizzi, and Barbosa Neto, 2005). Characterizing role of leaf health in yield and component traits, however, could provide a different perspective.

An effective photosynthetic surface area, especially that of uppermost leaves, is the most important assimilate contributing organ for the filling of grains. Therefore, a range of conditions that affect leaf health may have an implication in yield determination. Specifically, the stay green trait(indicative of leaf longevity and expressed as decline in photosynthetically active chlorophyllous surface), the foliar-disease resistance trait(expressed as levels of necrotic or chlorotic appearance), the leaf area index(LAI), the leaf waxyness and glaucousness, and other traits associated with the canopy structure that potentially predispose leaf to environmental factors, all of which prolong post-anthesis photosynthetic activity might be traits determining crop’s field level yield.

E. Statement of the problem

Due to the intrinsically heterogeneous nature of the land, breeding materials are often tested for their effects with limited power. A rather new spatial experiment design framework suited for early generation varietal trial, which accommodates large number of treatment entries in the study, could provide an efficient alternative to traditional block designs in estimating true genotypic effects.

Post-anthesis foliar health could be a proxy for determining final grain yield. It has a direct implication on grain filling behavior of a plant. Assessment of leaf trait offers an advantage in that these are but only largely affected by a few attributable environmental factors, such as temperature or soil moisture these traits (Joshi et al., 2007). Ideally, the contribution of pre-anthesis photosynthetic reserves to the final grain yield has been found to stall around 5% to 10% (Sharma, 1992). A major fraction of total yield, therefore, is dependent upon the photosynthesis that occurs post-anthesis. On the other hand, the yield synthesis post-anthesis is mainly a derivative of leaf area duration, which in turn is a function of leaf area index at anthesis and leaf longevity (Bingham, 1969). Terminal leaves, particularly the flag leaf and the one directly below it, have significant contribution to genotypic variation in yield(Aslam and Hunt, 1978) with respect to provisioning of assimilate to functionally active parts of plants including the storage organ(grain). This variation has been ascribed largely to the difference in the leaf longevity (Lupton, 1972).

Similarly, it is well known that diseases sustain reduction in yield with pathways that relate to the effective photosynthetic surface area (Lopes and Reynolds, 2012). Screening, and accounting for pathogen associated yield damage is usually difficult. But, a good indicator could be the pathogen inflicted foliar damage, which on the other hand can be as effectively quantified as yield (Sharma and Shrestha, 1994).

Spatial designs are a new avenue to field experiments. Their efficacies have not been extensively verified and thus the usefulness of information recovery they introduce through blocking. Current field testing will employ this technique in studying traits that contribute to post-anthesis photosynthetic efficiency and finally to the grain yield.

F. Literature Review

Use of unreplicated designs in field trials

Several authors have described experimental designs that can efficiently estimate the error variance in unreplicated trials. Some of the well-known proposed augmented designs and variants of it include randomized complete block and resolvable incomplete block designs for addressing one way heterogeneity, early row-column designs consisting of equal number of rows and columns defined with systematic arrangement of treatments including that for checks (Federer, Nair, and Raghavarao, 1975; Lin and Poushinsky, 1983).

In general, two common variants of augmented designs exist: Augmented block design and augmented row-column design. The former class of designs are useful in eliminating heterogeneity in one direction (in which blocks are laid), while those of latter class are effective in eliminating in two or more way field heterogeneity. Our study concerns the latter class of design.

Augmented row-column design for small number of check varieties.

Improvements have already been made in early block form of augmented designs in the past. With the description of row-column designs in early half of 1970, modification of original systematic-treatment-placement designs such as augmented lattice square (Federer and Nguyen, 2002), and α - α designs(Williams and John, 2003) were introduced. Augmented row-column designs for small number of checks were, however, a much latter addition. This class of augmented designs are more flexible, in that they allow for arbitrarily large number of new cultivars, the number of rows and columns, and even allow for dealing with additional form of three way field heterogeneity.

Design principles

Following design parameters are required to initialize a search for an efficient row-column design:

Number of rows: k

Number of columns: s

Number of checks: v_c

Number of rowgroups: g_k ($rg=1,2,\ldots,g_k$)

Number of colgroups: g_s ($cg=1,2,\ldots,g_s$)

Number of new entries: v_e

Number of plots allocated to checks per block: $v_{g_k g_s}$

(Piepho and Williams, 2016) has provided a framework for generation of this form of design. The steps are mentioned as follows:

1 Blocks are the units formed by the intersection of adjacent rowgroups and colgroups ($g_k g_s$). Define the number of plots for each block ($(g_k . g_s)_{rg.cg}$) that are to be allocated to checks. Note that, this number need not be a constant.

2 Allocate check cultivars (v_c) to check plots based on the following model for design factors: $g_k + g_s + g_k . g_s$.

3 In each row group (i.e., g_k), consider the classification of rows (k) by column groups (i.e., g_s). From step 2, we have a design for v_c -by- g_s classification. Consider both check cultivar and g_s of this design as factors and optimize the allocation of rows so that effects for rows can be estimated with good efficiency. This produces a row-column group design for check cultivar.

4 In each column group (i.e., g_s), consider the classification of row groups (i.e., g_k) by columns(s). From step 2, we have a design for the check v_c -by- g_k classification. Consider both check cultivar and rowgroups of this design as block factors and optimize the allocation of columns so that effects for columns can be estimated with good efficiency. This produces a row group-column design for check cultivars.

5 Merge the two designs obtained in steps 3 and 4 to obtain the final design for checks.

6 Allocate entries to free plots in completely randomized order.

Statistical analysis

The responses sampled from a row-column design may be analyzed using linear models with effect for both the row and column factors and, effects for genotypes. Further effects (rowgroup, columngroup, and rowgroup-columngroup interaction) that are associated with block also need to be specified, due to restricted randomization imposed in allocation of checks within blocks. Thus, the full model for a response y can be stated as:

$$y = \mu + rowgroup + colgroup + rowgroup.colgroup + rowgroup.row + colgroup.col + genotype + e$$

Where, μ is the common intercept and e is the residual plot error.

All block effects, with exception of effects for genotype, may be taken as random. Furthermore, the linear model may also be adjusted to fit a separate terms for fixed effects for checks and a random effects for new entries.

Spatial analysis in field trials

(Snedecor and Cochran, 1967) has cautioned that variables being studied be normally distributed and be spatially independent in order for popular statistical frameworks to be applied for inference. However, the underlying mechanics of natural environment ensure that factors like soil properties and crop yield components, instead of having random spatial distributions, do have spatial dependence, meaning that the observations are somehow related to their neighbors (Plant 2012; Van Es and Van Es, 1993). It seems obvious that the existence of spatial dependence is scale dependent. (Wendroth et al., 1997) found spatial dependence for soil fertility properties, and studies suggest its effects within experimental plot as close as 30 by 30 m (Vieira and Paz Gonzalez, 2003). Similarly, soil-atmosphere phenomena such as soil temperature are reported to be spatially autocorrelated at very high geographical resolutions (Al-Kayssi, 2002).

Factorial designs flexible enough to accommodate large number of treatments in a very compact space are among the latest developments in design of experiments. An augmented row-column design may be analyzed considering spatial model for plot error (e) (Clarke and Stefanova, 2011). A linear mixed effects model with spatial covariance structure specified for plot error (e) may be fitted. Spatial models assume that variables having spatial autocorrelation can be adjusted with some decreasing function of spatial distance, which usually results in improved precision of the analysis (Piepho and Williams, 2010).

Crop development and yield formation

Process attributing to yield formation, mainly growth and phenological processes, transcend the level of inputs and agricultural sophistication as evidenced by remarkable similarity of trend in yield despite differences in average yields across different countries for the past century (Satorre and Slafer, 1999).

Crop development is defined as the sequence of phenological events conditioned by external factors resulting in changes in the morphology and/or function of some organs (Landsberg, 1977). Internal or external morphological changes can, thus, describe the crop development. Duration of each phase of development and the developmental pathway of each subsequent phase, which is dictated by the initiation of primordial structures during earlier phase, is determined by the interaction between genetic and environmental factors. Among several methods those used to describe major developmental events, some which are non-destructive in sampling are also in use, one of such being the growth scoring code postulated by J. C. Zadoks (Zadoks, Chang, and Konzak, 1974).

As complex a trait the crop yield is, it is explained well when explored in relation to developmental phase of crop. Dynamics of multitude of factors are involved since the very beginning of a life cycle of crop, as early as the beginning of embryonic activities when inside the seed.

Leaf development

By the time wheat crop has grown to a seedling, five to seven leaf primordia will have been developed(McMaster, 1997), meaning that two to three leaf primordia will have already developed well before the tip of the first leaf appears. Duration through which the apical primary primordia persists depends on genotype and environmental factors. Plastochron (thermal time between the initiation of two consecutive leaf primordia) is usually a constant value for a given environment (Wilhelm and McMaster, 1995). The maximum number of leaves is determined when the apex changes the development from vegetative to reproductive with the floral initiation. A model devised by (Kirby, Appleyard, and Fellowes, 1985) relates leaf and spike initiation phases with number of leaves that appear in main shoot.

Tiller development

The phytomer contains axillary tiller buds, each of which is able to differentiate into leafy tillers. The relationship between the number of phyllochrons and the number of primary tillers that appear approaches perfectly linear, with an intercept at 3 units in horizontal axis when plotted the number of primary tillers approximated by phyllochrons. Similar pattern follows in tillers that develop from secondary and tertiary tillers. This relationship is only valid only to the extent provided resources are unlimited, in field, however, the potential tiller emergence rate is slower than that predicted by Fionacci series (Masle, 1985), as competition occurs within a single plant system and also between multiple of those.

Alongside the emergence and growth of the tiller, in environmental conditions, tiller mortality is also a defining factor for overall crop stand due maturity. This occurs in the order reverse to that in which the tillers appeared. Phenomena of tiller mortality is reasoned to accelerate at around stem elongation phase due concomitant increase in demand of assimilates to the developing shoots.

Spikelet and floret development

Spikelet initiation begins with the appearance of double ridge in the apical region of a shoot. This is followed by the floral initiation and finally terminates with the initiation of terminal spikelet in the apical meristem. It is also shown that the rate of spikelet initiation is negatively correlated total number of leaves on a shoot.

The floret development progresses towards the ends starting with the signs first visible in the lower middle portion of a spike head. Similarly, accumulation of the Growing Degree Days (GDD) provides an indication of for how long floret initiation within each spikelet continues. It continues approximately until a GDD period of $200-300^{\circ}$ C above 0° C, with the latter being reference temperature, before the appearance of the flag leaf ligule. It is postulated that floret death too occurs due to increased competition for assimilates, once stem and spikes growth surges, alike tiller death. The competition phenomena that determines the proportion of florets liable to maintain normal rate of development has been validated in studies that compared the genotypes having different partitioning between spikes and stems ratios (Brooking and Kirby, 1981).

Grain filling

Wheat is a kleistogamus plant. So, once the pollination occurs inside a floret, it begins to develop as a potential grain after it has been are fertilized.

Grain development in wheat passes through a series of distinct well characterized phases. In the initial “lag phase”, the grain does not gain much in weight. Although the phase generally spans almost $1/4^{th}$ of the total postanthesis period, only 5 to 10 percent of total grain weight is gained during this. However the term lag phase ascribed to earlier phase, the latter stage of grain growth– which substantially adds to the grain weight, can be well modelled both by in a logistic function as well as bilineal function.

This stage of development too is strictly regulated by the thermal time, in absence of severe environmental stresses however, in which case premature termination of grain filling leads to sub-optimal dry weight(Nicolas, Gleadow, and Dalling, 1984).

Crop yield and yield component traits

In modern wheat, gains in yield increases are due to increased harvest index, shorter period from sowing to anthesis, and more number of grains per unit area. Therefore, spikelet initiation and formation stage, which occurs before anthesis, and has direct relation to yield. This asserts that total number of grains/kernels per spike head (a component trait of yield) is largely a function of pre-flowering vegetative stage. It has also been concluded that genetic yield increases resulted from an increase in the number of kernels produced rather than an increase in kernel size (McCaig and DePauw, 1995). This suggests that bread wheat has been sink-limited at grain filling.

From the physiology perspective, yield component trait (i.e., for wheat, plants per unit area, spikes per plant, grains per spike, and grain weight) approach to understand yield is one but largely unreliable approach (Slafer, Calderini, and Miralles, 1996; Fischer, 1996). Owing to the fact that individual yield components are negatively correlated with each other, improvement in one trait will inevitably result in compensative negative effects in some other (Slafer, 2003). But understanding of exact nature of relationships between component traits might, enable breeders to manipulate them correctly (Slafer, Calderini, and Miralles, 1996). However, given that there exists a strong negative correlation among mostly vegetative stage defined yield component traits (summarized here as the number of grain kernels), it might be reasonable to seek to manipulate a different component trait such as grain yield. The grain weight is probably a much a simpler component, considering its distinctness from other correlated yield component traits in the pathway to yield determination; all other component traits could be summarized to number of grains for given unit of measure(usually, per unit land area).

Factors affecting wheat growth and development

Major influencer of wheat growth and development cycle is the temperature. Every stage of development is, however, to a different degree depending upon various factors (G. Slafer and Rawson, 1995), sensitive to temperature. This is unlike most other environmental growth affecting factors including moisture and photoperiod, which only exhibit their effects either when factors prevail at their extremes or are range in their effects based on the crop's growth stage.

Temperature

Generally, with higher temperature, the rate of development is faster and thus certain phase of crop growth becomes shorter. From among various temperature dependent growth models proposed to predict the growth phase, the thermal time model is the one most widely accepted (Squire et al., 1984). The simplified form of the model is shown below.

$$¿$$

$$mean\,daily\,temperature - base\,temperature\,\frac{\left(\square^{o}C\right)}{growth\,rate\left(kg\,da\,y^{-1}\right)}$$

$$thermal\,time = ¿$$

Conceptually, thermal time is the inverse of change in rate of growth rate per unit temperature, from base to optimum temperature. The concept assumes that the relationship between rate of development and temperature in linear. Similarly related are the leaf initiation and appearance and, the temperature. Empirically, the slope of the relationship is the rate of these processes per degree day, and the reciprocal of these slopes are the plastochron and phyllochron (Slafer and Rawson, 1995). So, at the least, the parameters of thermal time are specific for a given genotype and developmental stage. This way, the model can be linearized, as it remains unaffected by nuances of relationship change caused solely due to phasic development. In practice, however, thermal time is calculated as the accumulated daily mean temperature above a base temperature (Rawson and Bagga, 1979; Angus et al., 1981). Studies have highlighted the role of mean air temperature during booting and ear emergence (Calderini et al., 1999; Calderini et al., 2001). In a different study aimed at understanding the relationship between temperature dependent growth processes and natural variation associated with the temperature, mostly those conspicuous during vegetative to reproductive transition of wheat crop, authors investigated the vernalization requirements among Nepalese wheat and barley. Wheats from villages of high altitudes tended to require more vernalization than those from lower altitudes suggestive of local adaptation. This also leads to inference that seed exchange among Nepalese farmers is either poor or well provisioned with a sound knowledge base about local varietal adaptation (Rao and Witcombe, 1977).

Unlike atmospheric temperature, which is mostly influenced by incident solar radiation, Soil or root zone temperature is influenced several factors. The factors include intensity, the quality, and the duration of solar radiation, air temperature, surface vegetation, and the color and the thermal conductivity of the soil. The optimum soil temperature reported for maximum yield of the Bread wheat is $20^o C$ (Whitfield and Smika, 1971).

The cardinal air temperature for growth of wheat is summarized below:

Table of cardinal of temperature of wheat growth (Briggle and Curtis, 1987)

	Temperature
Minimum temperature	$3^o C$ - $4^o C$
Optimum temperature	$25^o C$
Maximum temperature	$30^o C$ - $32^o C$

Soil moisture

Water has its role in translocation of mineral and anabolic reactions inside plant system. Both high and low levels of moisture can reduce the crop yield, as excess moisture, above all, reduces soil aeration and thus the supply of Oxygen to roots. This can, in turn, have negative effect on soil microbial dynamics. Moisture stress causes both the reduction in cell division and cell elongation, thus in overall growth.

Wheat can be grown in regions where annual precipitation ranges from 250 to 1750 mm. Water associated stress has detrimental effect in all yield associated components (Abayomi and Wright, 1999). In a study that correlates effects of different levels of soil water tension measured near surface, authors point out that component traits like number of spikes per square meter, the number of spikelets per spike, and the number of grains per spike are among the most affected by increasing levels of soil-water-tension (Zhang and Oweis, 1999). Root characteristic is mostly associated with the genetic variation that confers drought resistance (Hoad et al., 2001).

Wheat grown in medium textured soil is unlikely to have simple moisture deficit (occurs when root zone profile exhausts of available water) during early stage of crop growth since wheat is usually planted under favorable soil moisture conditions. However, more serious consequences are faced in grain yield when water shortage occurs, in general, after ear initiation. One of the conditions which emphasizes role of moisture stress is due tiller mortality, which is dependent on pre-anthesis water availability (Aspinall and Husain, 1970; Begg and Turner, 1976). The potential grain number which is directly related to the production of fertile gametes and fertilization is other most influential yield-determining process affected by moisture stress during critical periods of pre-anthesis and anthesis (Bingham, 1966). Similarly, post-anthesis drought causing a reduction in thousand grain weight has been described by some authors as other major determinant of yield (Asana, Saini, and Ray, 1958; Day and Intalap, 1970; Day et al., 1978).

Solar radiation

The intensity of solar radiation varies with the latitude and the season. Tropical to Sub tropical regions occurring within latitude $20^o N$ - $30^o N$ observe the highest incidence of solar radiation.

Solar radiation is converted through fixative cycles into biomass or the crop's usable produce. An empirical framework to describe the relationship between the absorbed Photosynthetically Active Radiation (PAR_A), the radiation use efficiency and the factors accounting for the canopy structure, which is represented by leaf area index (LAI) and the radiation extinction coefficient (k), is stated by Beer's law (Goyne et al. 1993). The relationship is depicted below.

$$PAR_A = PAR_0\left[1 - e^{-kLAI}\right]$$

Where, PAR_0 is the above canopy radiation.

Radiation use efficiency is the slope of a linear function having dry matter accumulation per unit time, at the rate unconstrained by stress causing extraneous factors like soil moisture, nutrients, temperature, and the number of actively growing points, as response approximated by the absorbed photosynthetically active radiation (PAR_A).

As of the wheat, it is not the most efficient utilizer of PAR_A, with only 3.7% of total incident radiation utilized for dry matter accumulation. Sparing the energy efficiency factor, wheat has, however, a better growth rate compared to the C_4 plant Corn, reported when compared across similar growing conditions of temperate region (Fageria, Baligar, and Jones, 2010). In wheat, most of the between cultivar differences in growth duration is accounted for by photoperiod and vernalization sensitivity factor associated with the genotypes (Satorre and Slafer, 1999). Besides that, there is general agreement that a variety should have a high leaf area index and erect leaves, so as to make the best use of incident solar energy.

G. Rationale / Justification

Popular blocking designs offer limited advantage if sources of variation act in more than two directions. More importantly, as blocking is preconceived and immutable structure, further sources of variabilities aside from those addressed in design should be addressed via unconventional methods. Current study addresses multi directional heterogeneity issue through covariate adjustment technique, which as of recent is based on robust statistical procedures.

Significant time is invested by breeders and researchers often brooding on measures to manipulate yield. Yield estimates are dependent on the upon harvest yields, after completion of a crop cycle. This essentially is time intensive, and often futile with several detrimental factors looming throughout the crop cycle. A method of yield prediction, based on post-anthesis health of assimilate contributing system is a safeguard against potential adversaries of the crop. Similarly, in the current study, estimating effects of environmental factors on yield with the help of leaf health profile will be anchored by growth stage based interpretation; this method will render unnecessary to study the compensative nature of yield components.

Rationale has following missing components:
- How the site/region of testing is a proper choice for location.
- How author is competent to carry out research.
- How will this lead to further work in field experiment.
- How researching the crop and immediate outcome that derive from it is applicable to national as well as global community?

H. Research Questions

- Does field spatial variation have effects in vegetative and reproductive traits?
- Do genotypes with certain leaf characteristics yield better than others?
- Does the post-anthesis leaf health have yield predictive power?

I. Research Hypothesis

- Field spatial patterns have significant effects in vegetative and reproductive traits.
- Genotypes with certain leaf characteristics give better yields than others.
- Genotypic variation in yield and yield component traits can be explained by post-antheis leaf health.

J. Research Design and Methodology

Dimensions of the field
Total length of column: 19.5m
Total length of row: 18m
Individual plot size: 0.94 m^2
Total Area: 351 m^2
Net Plot Area: 225 m^2

Design, treatments specification and layout
Design type: Augmented row-column design

Number of rows (k): 12

Number of columns (s): 20

Number of checks (v_c): 4

Number of rowgroups (g_k ($rg=1,2,\ldots,g_k$)): 3

Number of colgroups (g_s ($cg=1,2,\ldots,g_s$)): 4

Number of new entries (v_e): 104

Number of plots allocated to checks per block ($v_{g_k g_s}$): *varies*

An augmented design that accommodates $v_e=104$ unreplicated entries and $v_c=4$ replicated check cultivars with the number of rows $k=12$ and the number of columns $s=20$ has been generated. Each row and column is a complete block with respect to checks. A block ($v_{g_k g_s}$) specified by intersection of a rowgroup and a colgroup will contain complete but varying number of check plots. A field layout of the randomized design has been depicted below:

Study will initialize with a small quantity seed of the treatment entries, and with only a small number of check varieties as this suits the problem domain being explored– application of row-column design for smaller number of checks.

The trial will be conducted under the condition of natural biotic factors influence; application of insecticidal sprays and disease checking sprays will be avoided in general. However, in case serious insect outbreaks (potentially Aphids and Armyworms) shall occur, routine sprays of relevant pesticides will be warranted. Observations on disease severity will be noted with special reference to Zadok’s scale of growth scoring. Since the study need to account for natural senescence in the genotypes being tested, Zadok's stage with score of only up to S77 (Late milk stage) will be investigated for changes in leaf senescence pattern. This is to acknowledge the fact that, after this stage the natural cycle of senescence would confound the effects that followed due to pathogen activity (Neupane et al., 2007). Forthcoming, the diseased leaf area might not always be distinguished clearly from the natural senescence.

Irrigation will be limited to once only throughout the growing period. This is considering that, the field will have ample amount of residual moisture during the period while crop establishes to start with, because the site usually stays logged with water during the months of August and extended halves of September. The irrigation will be scheduled to best avoid pre-anthesis moisture stress, as this has been implied in largest losses resulting in number of fertile florets and the final grain weight (Innes and Blackwell, 1981). Water will be sourced from a deep tubewell and the field will be moistened until saturated field condition will have been achieved.

The dominant soil type in the study site is a medium to heavy textured loamy soil.

Statistical analysis

For the design framework proposed, procedure for statistical analysis of response has been outlined earlier in statistical analysis of augmented row-column design for small number of check varieties section.

Planting and management

Expected Date of sowing: Nov 24, 2016

Standard agronomic practices recommended for normal fertility maintenance will be followed. Full rates of K2O and P2O5 will applied at the time of sowing. Nitrogen will be applied in split doses– 60 kg N ha^{-1} as basal and remaining 60 kg N ha^{-1} top dressed after irrigation.

Fertilizer amount allocation for whole field (on net plot basis)

Basal: 1.35kg N (2.93kg Urea) 1.465kg P2O5 0.45kg K2O Top dressed: 1.35kg N (2.93kg Urea)

Planting distance

Within rows in a plot, seeds will be sown continuously. A row spacing of 25cm in between distance will be allowed. A depth of 3-5cm below the surface will be the expected below soil surface distance for seed placement.

Intercultural operations

From previous experience, weed management is expected to be concerning. Particularly, *Rumex spp.* and *Chenopodium spp.* are suspected to claim the space. So, weeding operations at least twice, interspersed throughout the growing season, to check growth of any weeds will be undertaken.

Sampling procedure

For plant's growth, morphology and yield related observations, five tillers will be selected at random for observation of each trait from the population of plants in a plot (experimental units totaling to 240). A representative block of inner row will be prioritized, over the plants bordering the margins of a plot. While sampling observations for soil and atmospheric conditions, each plot will equally be partitioned to at least five sub-blocks and observation will be carried out on those sub-blocks. Standard procedure will be followed while recording canopy temperature depression as outline in Lopes and Reynolds (2012). For the measurement of thousand grain weight, arbitrary number of seed kernels ranging from 500 to more will be chosen randomly and counted. Their weights will subsequently be converted in to the grain weight of thousand kernels.

J.1 Research Method (Tick one) √		
Qualitative ( )	Quantitative (√)	Combined ( )

J.2. Study Variables

Data	Expected stage
Yield, morphology, phenology, soil and atmospheric conditions	
Seedling emergence	when approximately 50% of all plots' seeds are visible
Days to booting	when approximately 50% of the plants in a plot are at Zadoks stage 45
Days to heading	when approximately 50% of the plants in a plot are at Zadoks stage 55
Days to anthesis	when approximately 50% of the plants in a plot are at Zadoks stage 65
Days to medium milk stage	when approximately 50% of the plants in a plot are at Zadoks stage 75
Vegetative growth progression	Tillering (Zadoks stage 21-29)
Insect foliar damage score	Tillering (Zadoks stage 21-29)
Soil moisture temperature and EC	Jointing[^1] and Booting[^2](Zadoks stage 37 and 40)
Chilling injury	Heading (Zadoks stage 55)
Canopy sparseness	Heading (Zadoks stage 55)
Leaf glaucousness	Anthesis (Zadoks 60)
Canopy temperature depression	Anthesis (Zadoks stage 65)
Leaf chlorosis	Anthesis (Zadoks stage 65)
Leaf necrosis	Anthesis (Zadoks stage 65)
Leaf health	Anthesis (Zadoks stage 65)
Soil plant analytical development	Anthesis and medium milk(Zadoks stage 65 and 75)
Leaf area	Medium milk (Zadok stage 75)
Plant height	Medium milk (Zadok stage 75)
Weed score	Medium milk (Zadok stage 75)
Number of effective tillers	Medium milk (Zadok stage 75)
Leaf health	Medium milk (Zadoks stage 75)
Leaf senescence	Soft dough (Zadoks stage 85)
Defective heads count	Ripening (Zadoks stage 90)
Panicle length	Ripening (Zadoks stage 92)
Days to maturity	Ripening (Zadoks stage 92)
Number of grains per panicle	After harvested
Thousand kernel weight	After harvested and dried
Grain yield	After harvested and dried

J.3 Type of study (Tick one) √ and specify

Descriptive study ( )

Analytical study ( )

Experimental study (√)

Other ( )

J.4 Study Site and its Justification

The experiment will be undertaken at agronomy Farm of Agriculture and Forestry University (AFU), Chitwan, Nepal.

Particulars	Detail
Institution	Agriculture and Forestry University
Farm	Rampur
State	Chitwan
Longitude	84.4^o
Latitude	27.62^o
Altitude	191 meters
Environment	ME1
Dominant Soil(FAO classification)	Cambisols (Dystric Cambisols)
Surface pH	5
Cropping system	Rice-wheat

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J.5 Target Population

J.6. Sampling Methods (Tick one) √ and specify

Non-probability Sampling ( )

Probability Sampling ( )

J.7. Sample Size

J.8. Sampling Frame (if relevant) and Sampling Process including Criteria for Sample Selection

J.9. Tools and Techniques for Data Collection

A portable handheld SPAD meter and an infrared thermometer will be used for recording the leaf chlorophyll content of at minimum four flag leaves per plot and the canopy temperature (CT_a)during anthesis, respectively. The grain will be harvested manually in batches based on the date of maturity (defined by Zadoks stage 92-95). Similarly portable handheld digital tensiometer will be for measuring the soil moisture, temperature and electrical conductivity (EC). Alcohol thermometer, ruler, weighing balance will be used respectively for ambient temperature, length and weight measurement. Activities involving computer simulation will be performed with statistical packages (open sourced) in Windows or Debian platform.

J.10. Pre-testing the Data Collection Tools (if relevant)

K. Validity and Reliability of the Research (if relevant)

L. Biases (if relevant)

- Chances of over- or under-estimating effects for unreplicated entries due to human bias.

M. Limitation of the Study (if relevant)

- Crop losses associated with unpredictable weather patterns are not accounted for.
- Opportunistic infestation by insects and plant disease outbreaks, potentially resulting in heavy losses, will be out of scope of the study.
- Study assumes that experimental design is optimized well for the kind of problem being studied.
- Study is limited to only making inference on a narrow ecological scale, related to the study site, due to the nature of unreplicated trial which makes genotype-in-a-environment effects inestimable.
- Yield predictions entirely based on post-anthesis leaf health indicators might be unreliable when factors other than those encountered in current study are in play.

N. Plan for Supervision and Monitoring

O. Plan for Data Management

P. Expected Outcome of the Research

- Effects of major environmental factors in post-anthesis leaf stress will be estimated.
- Association of post-anthesis foliage stress to yield and yield component traits will be established.
- Influential adaptive traits for improving leaf longevity will be identified.
- Extent of spatial correlation among traits will be estimated.
- Based on overall performance of genotypes, recommendation for advancement into further generation will be

Q. Plan for Dissemination of Research Results

- Consulting with plant breeders, researchers, and academic community
- Publishing information to farmers involved in wheat production